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Title: PATHOLOGY, TISSUE RESIDUES AND VIRUS-INDUCED MORTALITY IN MICE EXPOSED TO LEAD AND CADMIUM

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Lead (Pb) and cadmium (Cd) are naturally occurring heavy metals which have been redistributed to a large extent by man in the environment. These metals have certain common toxic sites of action on biological systems of mammals. Simultaneous exposure to Pb and Cd is highly probable due to common sources of exposure in the environment. The purpose of this study was to observe the effects of simultaneous, chronic exposure to Pb and Cd on pathology, tissue residues and susceptibility to viral challenge in mice. Mice were exposed, via the drinking water, to either Pb or Cd or both metals simultaneously at various doses for ten weeks and then challenged with encephalomyocarditis virus (EMCV). Mortality was enhanced in all groups of mice exposed to Pb and suppressed in groups of mice treated with Cd. Mice simultaneously coexposed to Pb and Cd had mortality rates intermediate to those observed after exposure to the individual metals. It is proposed that Pb suppresses immunity in mice by inhibition of basic metabolic functions dependent on sulfhydryl-

containing enzymes. The effect of this action results in a decreased ability of cells to produce antibodies, interferon and anti-viral proteins. It is postulated that Cd enhances the immune response in mice by inhibition of zinc-dependent enzymes such as peptidases and RNA polymerase. This results in increased stability and persistance of antibodies, interferon and anti-viral proteins in addition to decreased ability of the virus to replicate. Coexposure to Pb and Cd did not appear to alter histopathologic lesions observed by light microscopy that were produced by exposure to Pb or Cd only. Pb and Cd residues in tissues of mice exposed to these metals, as compared to residues in tissues of mice which received Pb or Cd only, indicate that interaction occurs in regard to metabolism, storage and excretion of these elements. Coexposure to Pb caused increased Cd accumulation at low doses. The effect was reversed at high doses and Cd tissue residues are reduced. Pb tissue residues, except in kidneys, were generally lower in mice coexposed to Cd. Kidney Pb levels increased in these mice. It is significant that chronic coexposure to Pb and Cd at subclinical doses alters certain effects produced by exposure to only one of the metals. Simultaneous exposure to low levels of Pb and Cd better simulate natural conditions of exposure in the environment.

Pathology, Tissue Residues and Viral-Induced Mortality In Mice Exposed To Lead and Cadmium

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I. LITERATURE REVIEW

Lead

Lead (Pb) is a naturally occurring element that has been mobilized and redistributed to a large extent in the environment by man. Processes such as burning coal, combustion of "leaded" gasoline and airborne effluents of smelters have resulted in widespread environmental contamination with lead (Haley, 1968). Other sources of lead in the environment result from commercial uses of lead compounds in printing, plastics, putty, plaster, paint, solder, ceramic glazes and batteries (Haley, 1968; Ziefield, 1964). Exposure of people and animals to inorganic lead occurs from inhalation of airborne lead and ingestion of foods and beverages contaminated with lead (National Research Council, 1972). Numerous incidents of lead poisoning result from consumption of "moonshine" whiskey (Morgan et al., 1966), foods stored in improperly glazed earthenware or cans sealed with lead solder (Chisolm, 1971a). Ingestion of paint chips, putty, plaster, colored magazine pages or lead contaminated soil (i.e. pica) are common causes of plumbism in children (Haley, 1968; Smith, 1964). Acute industrial exposures are more common in adults (Lilis et al., 1977). Lead poisoning in the past may have resulted from use of lead plumbing fixtures, wine vessels and cooking utensils (Green et al., 1976).

The normal adult or child daily intake of lead is estimated to be 0.3µg oral and 0.3-0.5µg inhaled (Green et al., 1976). Ninety percent of ingested inorganic lead is excreted unabsorbed via the feces (Kehoe, 1961) and 70-75% of inhaled inorganic lead is exhaled (Chisolm, 1971a). Absorption of airborne lead through the respiratory system is directly dependent on the particle size of the inhaled lead (Tomashefski and Mitchell, 1966). Most of the absorbed lead is stored in the skeleton, especially in long bones. However, when lead intake exceeds excretion rate and bone storage, residues accumulate in soft tissues (Kehoe, 1961). For adults, this level is estimated to be 1 mg Pb/day. The first measurable sign of lead intoxication is increased urinary ALA which occurs when whole blood residues of lead are above 30µg/100 ml (Haeger-Aronsen, 1971). Mild symptoms of lead poisoning can occur when blood levels range from 60-80µg/100 ml or greater (Chisolm, 1971a). Blood concentration of lead is a good indicator of general exposure but should not be used to determine the degree of exposure because lead accumulates rapidly in blood and then plateaus. Concentrations of lead in soft tissues, however, continue to rise until exposure is terminated (Kehoe, 1961). Additionally, lead may accumulate in soft tissues due to reabsorption from bone (Chisolm, 1971a).

Exposure to organic lead compounds such as the gasoline additives, tetraethyl (TEL) or tetramethyl (TML) lead results in a different clinical picture (Zavon, 1964). These compounds are absorbed readily through the skin or gastrointestinal tract, do not accumulate in the

blood or affect heme synthesis, and excretion occurs rapidly in the urine. The major site of toxicity of TEL and TML is the central nervous system (Saunders, 1964).

Eighty-five percent of all lead poisoning cases are reported between May and October (Chisolm, 1971a). It has been postulated that the high frequency of lead poisoning during the summer months is partially due to 1) enchancement of intestinal absorption of lead by ultraviolet light (Chisolm, 1964), 2) decreased excretion and increased toxicity of lead with higher ambient temperatures (Kehoe, 1961) and 3) dehydration and acidosis enhancement of lead toxicity (Kehoe et al, 1940). Absorption, excretion and toxicity of lead are also affected by vitamins E (Leavander et al., 1977) and D, calcium (Sorrell et al., 1977) and selenium (Rastogi et al., 1976).

Lead has been reported to adversely affect hematopoiesis, the urogenital organs (Chisolm, 1971a, b; Haley, 1968), the central nervous system (Chazoki et al., 1963; Morgan et al., 1966; Fazlullah and Ramamurthi, 1965), immunological competence (Treagan, 1975; Vos, 1977), mitochondrial structure (Chisolm, 1971a), and chromosomal integrity (Forni et al., 1976; Deknudt et al., 1977).

Lead toxicity produces microcytic hypochromic anemia (Berk et al., 1970; Griggs, 1964), that apparently results from inhibition of sulfhydryl-dependent enzymes involved in heme synthesis (Bonsignore et al., 1966). Decreased heme synthesis results in shorter erythrocyte life span and a decreased number of mature circulating red blood cells. Erythropoietic tissues respond by releasing immature red

cells (reticulocytes) into circulation (Chisolm, 1971a). Basophilic stippling of red cells is common in lead poisoning.

At least two enzymes in the heme synthesis pathway have been reported to be inhibited by lead (National Research Council, 1972). Delta-aminolevulinic acid dehydrogenase (ALA-D) inhibition causes an accumulation of delta-aminolevulinic acid (ALA), a pathognomonic condition which can be detected from elevated levels in the urine. Inhibition of heme synthetase (ferrochelatase) causes increased levels of protoporphyrin IX in the blood (Chisolm, 1971a; Lilis et al., 1977). Most body cells synthesize and utilize heme. Cytochromes involved in oxidative phosphorylation are heme-containing enzymes.

Lead may also interfere with porphyrin synthesis and result in chronic metabolic hypoxidosis and alteration of brain hemodynamics which in turn may be related to encephalopathy observed in lead poisoning of children (Pentschew, 1965).

Effects of lead on kidney tissue are mainly changes of epithelial cells in the proximal convoluted tubules (Biagini et al., 1977). Long term exposure may cause chronic nephritis (Morgan et al., 1966; Goyer, 1971). Damage to renal tubule epithelial cells impairs cellular energy metabolism and decreases reabsorption of amino acids, glucose and phosphates (Hammond, 1977; Goyer, 1968). The resultant hypophosphatemia can result in mobilization of phosphates from bone tissue, and lead stored in bone can be mobilized at the same time, which can increase lead levels in soft tissue (Chisolm, 1971a).

Various degrees of permanent nerve tissue damage and mental retardation result from acute exposures to lead (Goyer and Falk, 1974). Acute lead poisoning in adults can damage Schwann cell mitochondria and result in demyelination and peripheral neuropathy (Chisolm, 1971a). Encephalopathy may occur in children after acute exposure to lead (Chazaki et al., 1963). Lead impairs the permeability of capillaries resulting in edema and swelling in the brain which increases intracranial pressure and destroys brain cells (Pentschew and Garro, 1966). The molecular layer of the brain is most severely damaged in lead-induced encephalopathy (Penschew, 1965).

Increased mortality occurs in animals given lead and subsequently exposed to bacterial (Hemphill et al., 1971; Selye et al., 1966) or viral infectious agents (Gainer, 1974, 1977a, b,; Thind et al., 1977). It is postulated that lead-exposed animals are more susceptible to infectious diseases due to an immunosuppressive effect of lead. Inhibition of antibody synthesis may be one mechanism of lead-induced immunosuppression. Numbers of splenic lymphocytes producing either IgM or IgG antibodies were decreased in lead exposed mice (Koller and Kovacic, 1974; Koller et al., 1976, 1977). Neutralizing antibody titer to pseudorabies virus was lowered in rabbits given lead (Koller, 1973). The ability of B lymphocytes and macrophages to form erythrocyte-antibody-complement rosettes with sheep red blood cells was decreased in mice given lead (Koller and Brauner, 1977). However, macrophage phagocytic and digestive ability was enhanced in a similarly lead-exposed group of mice (Koller and Roan, 1977). Delayed hyper-

sensitivity, a T lymphocyte-mediated monocyte dependent reaction, was suppressed in mice given lead (Muller, 1977).

Cells infected with certain viruses may produce interferon (IF) which in turn initiates synthesis of a protein responsible for providing protection to an uninfected cell by preventing the virus from entering the cell (DeMaeyer et al., 1970). Increased incidence of mortality in mice exposed to lead-virus combinations may be due to decreased IF protection (Gainer, 1977a; Thind et al., 1977). Lead may interfere with 1) induction of IF-induced protein synthesis by inhibition of RNA synthesis or 2) the protective action of IF or IF-induced protein.

Cadmium

Cadmium (Cd) occurs naturally in the earth's crust at average concentrations of less than 0.5 ppm (Anonymous, 1971). This metal occurs mainly as greenokite, a cadmium sulfide, and is usually associated with zinc in ratio ranging from 1 Cd:500-1000 Zn (Cox, 1974).

One of the major uses of Cd is to electroplate or galvanize ferrous metals (Schroeder, 1965a). Thus, Cd is present in products such as wheel bearings, nuts, bolts, screws, jewelry, galvanized water pipes, sprinkler systems and nuclear reactor rods and shields (Cox, 1974). Some food poisoning outbreaks have occurred from use of Cd for plating containers used for food and drink and cooking utensils (Bonnell, 1965). Cd compounds are contained in numerous other materials such as paints (color pigment), plastics (stabilizer), batteries, TV tube phosphors, fungicides, pesticides, rubber products

(curing agent), fertilizers, coal, motor oil, gasoline, solder, cigarette paper, tobacco, newsprint, sheetrock, plaster, shellac, varnish, scotch tape, photographic chemicals and fire detection units (Flick et al., 1971).

The major sources of environmental contamination by Cd result from 1) release of Cd during smelting of lead, zinc or copper (Cox, 1974), 2) industrial emissions from metallurgic processes (Beliles, 1975) and 3) heating or burning of Cd-containing materials such as scrap metal, plastics, cigarettes, coal, gasoline, motor oil and tire treads (Cox , 1974; Flick et al., 1971). Other routes of exposure to man and animals result from leaching of Cd from galvanized or plastic water pipes (Schroeder, 1966) and treatment of crops with Cd-containing fertilizers and pesticides (Flick et al., 1971; Schroeder and Balassa, 1963). Many natural products become contaminated with Cd from redistribution of this metal in the environment by man. Cd residues have been detected in grains (wheat, corn, oats), vegetables, meat, (game animals, pork, beef, shellfish) and dairy products (Schroeder and Balassa, 1961; Flick et al., 1971).

Lethal oral doses of Cd for man are unknown but severe symptoms have resulted from ingestion of 10 mg (Fairhall, 1957). The Public Health Service standard for Cd content of municipal drinking water is 10µg/l (Dept. Health, Education and Welfare, 1962). Cd toxicity may result from either acute or chronic exposure (Flick et al., 1971). Acute toxicity is generally associated with inhalation of Cd

fumes emitted from heating material which contains Cd. Acute toxicity usually manifests as chemical pneumonitis (Beliles, 1975). Chronic Cd poisoning occurs from long term ingestion or inhalation of subclinical doses of Cd-containing material. Cd is absorbed more readily by pulmonary exposure (40%) than by ingestion (5-10%) (Friberg et al., 1971). The emetic properties of Cd are somewhat protective to humans in instances of ingestion of large acute doses (Flick et al., 1971).

Cd compounds have been implicated in adverse toxic effects on renal, skeletal, hepatic, reproductive, pulmonary, central nervous, hematopoietic and cardiovascular systems (Beliles, 1975; Vigliani, 1969; Friberg, et al., 1971). In addition, Cd has been associated with teratogenesis (Patton and Allison, 1972; Shiraishi et al., 1972) and carcinogenesis (Potts, 1965; Gunn et al., 1967, Friberg et al., 1971). Cd also inhibits RNA, DNA and protein synthesis (Stoll et al., 1976).

Chronic ingestion of rice contaminated by Cd in irrigation water produced severe toxic effects to natives of the Jintsu River Basin in Japan (Tsuchiya, 1969). The disease which developed from this chronic exposure to low levels of Cd is called "itai-itai byo" (ouch-ouch disease). Typical symptoms associated with this disease are proteinuria, aminoaciduria, renal damage (Fukuyama and Kubota, 1970; Hagino, 1970; Nomiyama and Sugata, 1972; Iguchi, 1973) and severe skeletal disorders such as osteomalacia and brittle bones (Takase, 1967). A relationship may exist between renal and skeletal disorders produced by long term exposure to Cd. Originally it was postulated

that skeletal lesions such as osteoporesis or osteomalacia were secondary to effects of Cd on some other tissue (Friberg et al., 1971). This belief was based on data which showed Cd did not accumulate in bone tissue (Nordberg and Nishiyama, 1972). It was reported that Cd-induced damage to the proximal convoluted tubules in the kidney caused abnormal amounts of calcium and phosphate ions to be excreted in the urine. This resulted in a calcium deficiency and calcium mobilization from bone tissue, with resultant skeletal disorders (Belilles, 1975). More recent studies, however, reported that the bone lesions occurred prior to any apparent kidney damage (Yoshiki et al., 1975). Therefore, Cd may have a direct toxic effect on bone tissue unrelated to renal damage. Also, it has been shown that Cd exposure results in a thinning of compact bone in rats, however, no effects were observed in rats only deprived of calcium and not treated with Cd (Itokawa et al., 1974).

Proteinuria, which is characteristic of chronic Cd exposure (Piscator, 1962, Kazantzis et al., 1965), results from inhibition of Zn-dependent exopeptidases in plasma cells, reticuloendothelial cells and renal tubular cells which catabolize protein fragments (Vigliani, 1969). A major portion of the protein fraction found in urine of animals and people chronically exposed to Cd originates from light chain segments of immunoglobulin molecules. These protein fragments are not catabolized due to inhibition of the Zn-dependent aminopeptidases (leucineaminopeptidase) and carboxypeptidases (Vigliani, 1969). Proteinuria is enhanced by inhibition of reabsorptive mechanisms of

the proximal convoluted tubules of the kidney (Vigliani, 1969). The latter effect may be related to inhibition of sulfhydryl-dependent enzymes in the heme synthesis pathway. Ultimately this could cause a depletion of heme-containing enzymes (e.g. cytochromes) that function in energy metabolism needed for active transport mechanisms and other cellular functions.

Testicular necrosis and atrophy induced by Cd (Parizck, 1956) is also due to inhibition of Zn-dependent enzymes in the testes, and causes altered permeability of testicular vasculature, edema, and pressure changes that result in local ischemic anoxia (Kar and Das, 1960; Gunn et al., 1963).

Effects of Cd on pulmonary function can be severe. Acute inhalation of 50 mg Cd/M^3 for one hour can result in death from pulmonary edema-induced anoxia within three days (Friberg et al., 1971). Acute exposure may result in delayed effects such as perivascular and peribronchial fibrosis accompanied by coughing, bronchitis and emphysema (Paterson, 1947; Thurlbeck and Foley, 1963). Chronic inhalation of low levels of Cd can result in emphysema not preceded by a history of bronchitis and coughing (Beliles, 1975).

Cd exposure has also been implicated in such disorders as 1) cardiovascular arteriosclerosis, ventricular atrophy and vestibular disturbances (Kanisawa and Schroeder, 1966), 2) hepatic cirrhosis and fatty degeneration (Schroeder, 1965a; Schroeder et al., 1964), 3) hypertension due to renal arteriosclerosis and glomerular damage (Schroeder, 1964, 1965b) and 4) neurological disorders associated with hemorrhagic

lesions of sensory nerve ganglia (Flick et al., 1971) and olfactory nerve damage resulting in anosmia (Potts, 1965). Cd has also been shown to inhibit DNA, RNA and protein synthesis in rat liver cells (Stoll et al., 1976). These effects may be related to oncogenic properties of Cd observed in some animals.

The biological half life of Cd in tissues of man and animals is extremely long, ranging from 10 to 25 years (Cotzias et al., 1961; Matsubara-Khan, 1974). The main sites of Cd accumulation in the body are liver and kidney tissue (Syversen, 1975, Exon et al., 1977). The liver accumulates larger amounts initially, but if exposure is prolonged, kidney levels become greater. The renal cortex appears to be a specific site of Cd deposition in kidneys (Gunn and Gould, 1957). This preferential accumulation of Cd in the renal cortex may be due to an abundance of sulfhydryl groups or metallothionine located in that region (Cotzias et al., 1961).

Exposure to Cd compounds induces a de novo synthesis of a carrier protein, metallothionine, in the liver (Colucci et al., 1975; Kagi and Vallee, 1960). Metallothionine induction and its high affinity for binding Cd may be a protective to Cd toxicity by lessening its effect on other vital proteins (Vigliani, 1969). Cd is transported in the blood on this protein and is excreted mainly in the urine (Friberg et al., 1971).

Recent studies indicate that Cd adversely affects immune mechanisms in mammals. Cd was reported to enhance the incidence of mortality

induced by some infectious agents (Exon et al., 1975; Gainer, 1977b). In instances of virus challenge, Cd may reduce the action of either IF or an IF-induced anti-viral protein (Gainer, 1977a).

Circulating antibody (Ab) titers to pseudorabies virus were reduced in Cd-treated rabbits (Koller, 1973). In a study using rats, circulating Ab to human gamma-globulins were enhanced when Cd was given 14 days prior to the antigen (Ag) challenge but was reduced when Cd was administered 7 days before exposure to the Ag (Jones et al., 1973).

Ab responses to most Ag require cooperation between at least two types of lymphocytes for optimal expression (Miller et al., 1977). One cell type is thymus-derived (T cell or T lymphocyte). The other cell type is bone marrow-derived (B cell or B lymphocyte). T cells function as cytotoxic cells, as in cell-mediated immunity and delayed hypersensitivity, and influence the action of B cells, probably by production of some soluble factor which acts on the B cell. T cells do not produce Ab. B cells are responsible for Ab synthesis and differentiate into plasma cells in the presence of Ag stimulation to produce Ab against a specific Ag (Eisen, 1974). A third cell type is the macrophage which functions as an accessory cell by cooperating with T cells in cell-mediated immune responses and also aides the B cell response to Ag (Cohn, 1968).

The effects of Cd on specific cell populations responsible for eliciting the immune response have been studied. Peritoneal macrophages isolated from Cd-treated mice demonstrated increased phagocytic activity and increased acid phosphatase levels (Koller and Roan,

1977). The ability of B lymphocytes to form rosettes was decreased in mice chronically exposed to Cd (Koller and Brauner, 1977). Ab synthesis by splenic lymphocytes was significantly reduced in mice treated with Cd for 10 weeks. Ab synthesis remained suppressed for at least six weeks after discontinuance of Cd exposure (Koller et al., 1975). A greater inhibition of Ab synthesis occurred during the secondary or memory immune response, which was measured by synthesis of IgG, 7S Ab. Since the memory or secondary immune response is more dependent on T lymphocyte helper function than is the primary immune response (Anderson et al., 1974), it was postulated that the T lymphocyte may be a specific target of Cd toxicity. A subsequent study, now in progress, shows that mitogen stimulation of both T lymphocytes and B lymphocytes may be affected by chronic exposure to Cd (unpublished data). Another study reported that Cd effects on Ab synthesis were altered by the route of exposure to Cd when administered in single acute doses (Koller et al., 1976).

II. INTRODUCTION

The majority of research which deals with the toxicology of environmental pollutants involves studying the effects of a purified form of a toxicant on one or several species of animals. Results obtained from these studies are used to estimate the hazard the compound represents to human health. This type of research is initially essential to determine the type and site of toxic effects to be expected in the event of exposure of man or animals to the toxicant. In reality, however, people are simultaneously exposed to a variety of toxic agents in the environment. It is important to determine if the effects of specific compounds on biological functions are modified by the presence of, and exposure to, other environmental contaminants.

Interactions between some types of chemicals in biological systems have been documented. Drug interactions are most notable in this respect (Goldstein et al., 1974). Potentiation or antagonism of toxic effects of organochlorine and organophosphate pesticides due to simultaneous exposure is common (Dubois, 1969; Murphy, 1969). These latter interactions occur with enough frequency that federal agencies now recommend that interaction studies be included in toxicity screening of these pesticides.

Coexposures to certain heavy metals have been studied. Zinc can counteract or prevent some toxic effects induced by Cd (Flick et al., 1971). Toxic effects of both methylmercury and selenium are decreased when exposure to the metals occurs simultaneously (Ganther et al. , 1972) . Selenium has also been reported to decrease

arsenic (Holmberg et al., 1969), Cd (Kar et al., 1962) and Pb toxicity (Rastogi et al., 1976).

To my knowledge, the effects of coexposure to lead and cadmium have not been reported. Yet these metals commonly occur together in the environment and simultaneous exposure is likely. Cd and Pb occur together as natural ore deposits (Anonymous, 1971). Smelting lead simultaneously releases both metals into the terrestrial, atmospheric and aquatic environment around refineries (Anonymous, 1971). Both metals are released during burning of coal, motor oil and gasoline. Compounds of both metals are present in paints, solder, storage batteries, ceramics, glazing material, newsprint and many natural foods (Flick et al., 1971; Haley, 1968). In addition to common sources of both Pb and Cd, exposure to each metal could occur simultaneously from separate environmental sources.

Alteration of metabolism or action of a toxicant may be modified by the presence of a similar toxic compound through utilization of common metabolic pathways. Pb and Cd are classified as heavy metals. Heavy metals, in general, are known to have certain common toxic sites of action (Oehme, 1972). Pb and Cd both exert toxic effects on hematopoietic and nervous systems in addition to causing lesions in the liver and kidneys (Belilse, 1975). Each metal also has been reported to suppress immune responses in mammals (Hemphill et al., 1971; Koller, 1973; Koller and Kovacic, 1974; Koller et al., 1975, 1976; Selye et al., 1966; Muller et al., 1977). Because of the potential for simultaneous exposure to Pb and Cd in the environment

and their common sites of toxicity in the body, it is important to study the effects of coexposure to these compounds. The purpose of this study was to observe the effects of simultaneous chronic exposure to Pb and Cd on pathology, tissue residues and susceptibility to viral challenge in mice.

III. MATERIALS AND METHODS

Treatment Groups

One hundred and ninety-five weanling, male Swiss Webster/Sm mice were divided into 13 groups of 15 mice each (Table I). Eight groups of these mice were exposed to either 3, 30, 300 or 600 ppm Cd as cadmium acetate of 13, 130, 1300 or 2600 ppm Pb as lead acetate. Four groups of mice were exposed to Pb and Cd simultaneously at concentrations of 3 ppm Cd + 13 ppm Pb, 30 ppm Cd + 130 ppm Pb, 300 ppm Cd + 1300 ppm Pb or 600 ppm Cd + 2600 ppm Pb. The metals were given in deionized drinking water. Control mice received only deionized drinking water (Table I). The diet fed was Oregon State University Rodent Chow. Mice were housed in 29 x 19 x 13 cm polycarbonate cages with stainless steel lids. Alder shavings were used as bedding material and room temperature was maintained at 20-22^{o} C.

Virus Preparation and Inoculation

A stock encephalomyocarditis virus (EMCV) supply was passed through four groups of mice consecutively. Virus was collected from infected mice by removing the brain by sterile technique. Each brain was then homogenized using a Virtis "45" homogenizer. The dissociated brain cells were subsequently sonicated with a Tekmar Model SDT sonicator to release the virus from the cells. Supernatant from the sonicated brain cell mixture was collected by centrifugation for 20 minutes at 2000 x g and stored at -70^{o} C.

The 14 day LD_{50} of the virus solution was obtained by inoculating groups of mice with serial dilutions of virus. The desired dilution was determined to be $1x10^{-6.1}$ given in a 0.2 ml aliquot.

Data Collection

After ten weeks exposure to the experimental regimens, all mice were inoculated intraperitoneally with 0.2 ml of EMCV diluted in Hank's Balanced Salt Solution to $1x10^{-6.1}$. Mice were observed for 16 days after virus inoculation at which time the experiment was terminated. Mortality was not observed after day 13. Moribund mice in each group were necropsied and brain, heart, liver, kidney, lung, spleen and testes were collected, fixed in buffered formaldahyde, processed by standard techniques and stained with Harris' hematoxylin and eosin for microscopic study.

Lead-Cadmium Analysis

The same tissues as above were collected from three mice in each group, frozen in plastic bags and later analyzed for Pb and Cd residues using a Varion Model 1200 atomic absorption spectrophotometer by the method of Meranger and Somer (1968). At the time of analysis, tissues of each group of mice were pooled, weighed in tared ten ml beakers and placed in a 100^{o} C muffle furnace overnight. The temperature was slowly raised 25^{o} C/30min.to 500^{o} C and held overnight. The furnace was cooled to 200^{o} C and the samples were removed. Seven ml of 5% HNO_3 was added to the ash and the solutions

were allowed to stand for 15 minutes. The samples were analyzed by flame atomic absorption using a hydrogen continuum lamp for background correction. The 217.0 nm line was used for Pb and the 228.8 nm line for Cd. A neutral flame was used with both metals.

IV. RESULTS

Mortality

Mice exposed to lead (Pb), cadmium (Cd), or both metals simultaneously for ten weeks and then inoculated with EMCV yielded the following data: 1) Mortality was greater in all groups of mice exposed only to Pb than in controls or mice given only Cd (Table II). 2) Mortality was less in all groups of mice exposed to Cd than in controls (Table II). 3) In groups of mice given Pb and Cd simultaneously mortality was lower than in Pb-exposed groups, greater than in Cd-exposed groups (except at 600 Cd-2600 Pb) and slightly lower than in the controls.

Body Weight

In general, mice which received Cd, with or without coexposure to Pb had decreased body weights (Table III). Significant decreases in body weight ($P \leq 0.5$) occurred after six and ten weeks exposure to 600 ppm Cd or 600 Cd-2600 Pb and after ten weeks exposure to 300 Cd-1300 Pb. Mice given only Pb had body weights similar to controls throughout the experiment.

Tissue Analysis

Tissue concentrations of Pb or Cd increased as exposure levels of the metals were increased in the drinking water. Cd residues in liver and kidney tissue of mice given either Cd or Cd and Pb simultaneously (Table IV) were greater than Pb residues (Table V) even though the

dietary concentration of Pb was as much as four times that of Cd at comparable doses.

Pb concentrations in heart, liver, spleen, testes and lung were generally decreased in mice coexposed to Pb and Cd compared to residues in mice which received only Pb (Table V). Kidney Pb residues of mice coexposed to Pb and Cd were increased except at the 600 Cd-2600 Pb exposure level where Pb residues were decreased. Corresponding liver Pb residues were increased at that exposure level.

Cd residues were increased in tissues of mice coexposed to Pb and Cd at the three lower interaction levels (3 Cd-13 Pb, 30 Cd-130 Pb and 300 Cd-1300 Pb) compared to mice which received only Cd (Table IV). Cd tissue residues of mice simultaneously exposed to Pb at the high interaction dose were decreased compared to Cd residues in tissues of mice which received only the high Cd dose. Cd levels were greatest in kidneys of mice in the two lower Pb-Cd interaction groups (3 Cd-13 Pb and 30 Cd-130 Pb) and the two lower Cd only exposed groups (3 Cd or 30 Cd). However, liver Cd residues were greater in the two higher Cd-Pb interaction groups (300 Cd-1300 Pb aor 600 Cd-2600 Pb) and the two higher Cd-only exposed groups of mice (300 Cd or 600 Cd).

Pathology

Histopathologic lesions observed in kidneys of mice exposed to Pb or Cd, or a combination of these metals, manifested as moderate degeneration and necrosis of the tubular epithelial cells. Intranuclear

inlcusion bodies were observed in kidney cells of mice treated with Pb. Relative numbers of inclusion bodies appeared to increase in cells of mice exposed to the two higher doses of Pb. Megakaryocytosis was evident in spleens of Pb-treated mice. Histopathologic lesions, other than those induced by encephalomyocarditis virus in brain and heart tissues, were not observed in the remaining tissues by light microscopy.

V. DISCUSSION

It is likely that coexposure of mice to Pb and Cd produce different effects than result from treatment with the individual metals. The incidence of EMCV-induced mortality of mice after exposure to the individual metals was altered when mice were exposed simultaneously to Pb and Cd. Histopathologic lesions produced by Pb or Cd treatment did not appear to be altered by coexposure to the metals. Coexposure of mice to Pb and Cd did result in differences in tissue residues of the metals compared to mice treated with only one metal.

The effects of Pb on incidence of viral-induced mortality of mice in this study supports previous data which indicate that Pb exposure is detrimental to immune systems in mammals. Gainer (1977b) reported that Pb-treated mice were more susceptible to EMCV than non-treated mice. He postulated that Pb interefered with the induction, synthesis or action of interferon (IF). IF can be induced by a synthetic compound, Poly I/Poly C (PIC) and macrophages appear to be the site of IF synthesis (Field et al., 1968). Newcastle disease virus (NDV) also induces IF, but the site of IF synthesis is thought to be in lymphocytes (Woodruff and Woodruff, 1970). Gainer (1977a) studied the effects of Pb treatment on mice protected against EMCV infection by pre-treatment with NDV or PIC. He found that Pb abolished the protective effect of both NDV and PIC if Pb was administered prior to the IF inducers. However, the effect of Pb decreased or was absent if administered four to 24 hours after the IF inducers. Furthermore, a protective effect

of IF was not altered if presynthesized IF was injected into Pb-treated, EMCV-challenged mice. These results indicate that Pb affects the induction or synthesis of IF, but not the action of IF after it is synthesized This hypothesis was challenged, however, by data that indicate IF titers in brain tissue of Pb-treated, EMCV-challenged mice were not different from controls, yet the mortality incidence in these mice was increased (Gainer, 1977). The effect of Pb treatment on IF requires further investigation.

In another study, Pb treatment enhanced the incidence of mortality in mice inoculated with Langat virus (Thind et al., 1977). Decreased IF production and lowered neutralizing Ab titers were observed in these Pb-treated mice. Lowered neutralizing Ab titers were also observed in Pb-treated rabbits (Koller, 1972). Ab synthesis by splenic lymphocytes of Pb-treated mice was decreased (Koller and Kovacic, 1974; Koller et al., 1976, 1977). The secondary or memory immune response was more severly suppressed than the primary response in those studies. The authors suggested that the T helper-lymphocyte may be the target cell for Pb toxicity because the memory response is more dependent on T cell helper function than is the primary immune response. Further indications of effects on T lymphocytes were noted by suppressed delayed-type hypersensitivity in Pb-exposed mice (Muller, 1977). Delayed-type hypersensitivity is a T lymphocyte-dependent immune response. Work is now in progress to study effects of mitogenic stimulation of mitosis on T and B lymphocytes of Pb-exposed mice (unpublished data).

Adverse effects of Pb on T cells could affect all aspects of the immune response. These lymphocytes are essential for cell-mediated immunity (delayed-type hypersensitivity) and are also necessary for Ab synthesis (humoral immunity). T cells are believed to produce a soluble factor which aid the differentiation of B cells to plasma cells and regulate subsequent Ab synthesis (Eisen, 1974).

The mechanism of toxic action by Pb to cells involved with immunity is not fully understood at this time. The site of toxicity is likely at the biochemical level of cellular metabolism. Based on known mechanisms of Pb toxicity, (Chisolm, 1971) adverse effects on cells of the immune system could be the result of Pb-induced inhibition of enzymes. Pb is known to inhibit heme synthesis via inactivation of sulfhydryl-dependent enzymes of the heme synthesis pathway (Chisolm, 1971). An impairment of heme synthesis could decrease the activity of heme-containing enzymes such as cytochromes. These enzymes are essential for energy production within cells to carry out normal metabolic functions (Lehninger, 1970). Decreased cellular energy in cells responsible for immunocompetence could result in an inability to perform normal defense reactions such as Ab synthesis and active transport of humoral factors across cell membranes. Cytochromes are primarily located in mitochondrial membranes or endoplasmic reticulum. Morphologically, both of these cellular organelles are disrupted in liver and spleen cells of Pb-treated mice (Hoffman et al., 1972).

Impairment of the production of humoral factors or transport of these factors out of the T lymphocyte could result in detrimental

effects to both cell-mediated and humoral immune responses. A similar analogy could explain effects of Pb treatment on IF induction and release. Techniques are presently being developed to test T lymphocyte integrity in Pb-exposed mice. T and B lymphocytes of Pb-treated and non-treated mice will be collected, separated and cross-mixed to test for effects of Pb on each cell type.

The effects of Cd on immunity, as compared to the effects of Pb, may be more complex. Cd-treated mice in this experiment and two subsequent experiments (unpublished data), were protected against the incidence of EMCV-induced mortality. A previous study showed that Cd-treated mice were more susceptible to EMCV infection (Gainer, 1977b). It was thought that Cd may interfere with IF protection but in vivo tests failed to demonstrate any effects of Cd treatment on IF titers of EMCV-challenged mice (Gainer 1977a). In addition, no effect of Cd was observed on NDV or PIC-induced IF protection of EMCV-challenged mice. Cd has been reported to reduce IF protection in vitro, but only at very toxic concentrations (Gainer, 1977a). Exposure of Cd-treated mice to Rauscher leukemia virus did not affect the pathogenicity of the virus as measured by early erythroleukemic responses (Gainer, 1973). At present, only one study has demonstrated the Cd enchances EMCV-induced mortality in animals and other attempts to demonstrate mechanisms of action have been unsuccessful.

Recent investigations have shown that Cd treatment results in decreased Ab levels in several different species of mammals. Cd-treated

rabbits had decreased circulating neutralizing Ab titers to pseudo-rabies virus (Koller, 1973). Rats given Cd had lowered neutralizing Ab titers to human gamma-globulins (Jones et al., 1973). Ab synthesis by splenic cells of mice chronically exposed to Cd was markedly reduced, even six weeks after discontinuance of Cd exposure (Koller et al., 1975). As was the case after Pb exposure, Cd appeared to have a greater effect on the secondary or memory immune response. This indicates that the T lymphocyte may also be a target cell of Cd toxicity.

Studies of the effects of Cd on immunity in mammals appear contradictory. Cd treatment in the present study indicate a protective effect of Cd on the incidence of viral-induced mortality im mice. Yet numerous previous experiments showed that Cd reduced Ab synthesis in animals exposed to a variety of Ag including viruses (Koller, 1973; Koller et al., 1975, 1976; Jones et al., 1973). It is possible that Cd affects several metabolic pathways utilized in immune responses. Cd-treatment could cause decreased heme and Ab synthesis by inhibition of sulfhydryl-dependent enzymes as postulated above for Pb exposure. In the case of exposure to viruses, decreased Ab synthesis could be offset by enhancement of alternate defense mechanisms such as increased IF protection, increased Ab stability or interference with virus replication.

Chronic exposure to Cd results in proteinuria in man and animals (Vigliani, 1969). This effect is due to inhibition of Zn-dependent carboxypeptidases and aminopeptidases which catabolize proteins.

Proteins, such as IF, may be more persistent due to decreased activities of peptidases and thus intensify or prolong the protective action of IF to infection by a virus. It has been reported that peptidases decrease IF protection in vitro (Burke, 1977).

Protein fragments observed in the urine during Cd exposure are composed largely of light chain portions of immunoglobulins which are not catabolized due to decreased activity of Zn-dependent peptidases inhibited by Cd (Vigliani, 1969). Ab are composed of immunoglobulins (gamma-globulins), (Eisen, 1974). If inhibition of catabolism of fragments of these proteins occurs, a similar effect may occur in regard to intact Ab. Therefore, even though less Ab is produced, that which is produced may last longer because it would not be catabolized as readily.

Zn deficiency decreases the activity of RNA polymerase (Terhune and Sandstead, 1972). Many viruses depend on RNA polymerase for replication and synthesis of viral proteins within cells. Cd is a potent inhibitor of Zn-dependent enzymes (Vigliani, 1969). The presence of Cd in cells could simulate a Zn deficiency, thus decreasing RNA polymerase activity and reduce viral replication.

The results of this experiment demonstrate that an antagonistic interaction of Pb and Cd occurred in regard to the incidence of EMCV-induced mortality. Coexposure to Pb and Cd resulted in mortality rates similar to, but usually less than controls and intermediate between mortality incidence in groups of mice exposed to either metal.

Antagonism effects on the incidence of mortality could be explained on the bases of the individual activities of the two metals on immune systems which were discussed in the previous sections. Pb could enhance viral-induced mortality by interfering with the induction, synthesis or action of IF. Cd could offset the effects of Pb by increasing the stability and prolonging the action of IF, anti-viral protein and Ab, in addition to slowing down viral replication. The sum of effects resulting from simultanoeus exposure to Pb and Cd would produce results intermediate to the effects of exposure caused by each metal.

The data from this experiment support the proposed antagonistic interaction concept at the three lower dose levels of Pb and Cd. Groups of mice exposed to the highest level of Pb and Cd used in this study, however, reacted somewhat differently to virus exposure. Mortality incidence was increased in all Pb-exposed groups of mice, yet the least mortality (64%) occurred in the group that received the highest concentration of Pb. Exposure to Cd was apparently protective against EMCV infection in these mice, yet mortality incidence was greatest (40%) in the group of mice exposed to the highest concentration of Cd. Mortality in groups of mice coexposed to Pb and Cd at the three lower levels used in this study was always intermediate between groups of mice treated with either metal at comparable doses. Mortality in the group of mice coexposed to the highest level of Pb and Cd, however, was less than in either group of mice which were exposed to only one metal at the high dose. It appears that additional

factors other than those discussed previously may influence mechanisms by which high doses of Pb and Cd affect EMCV-induced mortality in mice.

A less pronounced increase in mortality incidence in mice exposed to the high Pb dose could be due to accumulation of high levels of Pb in certain tissues. Pb may accumulate to a point that levels become inhibitory to viral invasion of cells or inhibit some other action of the virus such as replication. Pb tissue residues were greatest at the high level of exposure (Table V), especially in the heart (5 ppm), which is a critical site of EMCV infection. Pb heart residues were decreased in mice coexposed to Cd at the high dose.

Increased mortality in mice exposed to 600 ppm Cd is probably related to a combined effect of Cd toxicity at this high dose and virus infection. Cd tissue residues are higher than in other groups (Table IV) and body weights were significatnly ($P \leq 0.05$) decreased (Table III), which is indicative of toxicity. Tissue residues of Cd at these levels may be toxic enough to begin to override the protective effect of Cd to virus infection seen at lower levels of Cd exposure. Thus, the incidence of viral-induced mortality would increase.

Cd residues were reduced in all tissues when Pb was given simultaneously (Table IV). Mortality incidence was markedly decreased in the group of mice exposed to both metals at high doses. The latter effect could be due to 1) reduced Cd toxicity due to decreased accumulation of Cd in the presence of Pb exposure, thus lowering Cd tissue residues and restoring the protective effect of Cd seen at lower levels of exposure and 2) reduction of Pb-enhanced viral-induced mortality

at the high Pb dose. Thus, the combined effects of these metals at high doses on EMCV-induced mortality could account for the greater reduction in mortality than occurs with coexposure to intermediate or low doses of these metals.

Pb and Cd residues in tissues of mice coexposed to these metals, as compared to residues in tissues of mice which received Pb or Cd only, indicated that some interaction may occur in regard to metabolism, storage and excretion of these elements. With certain exceptions, it appeared that concurrent exposure to Pb at low doses resulted in increased Cd accumulation in tissues of mice (Table IV). This effect was most evident in groups of mice exposed to the three lower levels of Pb and Cd used in this study. The effect was reversed, however, at the highest exposure level where coexposure to Pb resulted in decreased Cd residues in all tissues examined. The effect of Pb on reduction of Cd tissue residues at high doses may relate to decreased mortality in this group of mice as discussed previously.

Coexposure to Cd appeared to alter accumulation of Pb in some tissues. Generally, Pb levels were lower in tissues of mice coexposed to Cd (Table V). This was not the case, however, for renal Pb residues. Pb levels were increased in kidney tissue at all doses except 600 Cd-2600 Pb. Kidney residues of Pb in that group were decreased. The effect of coexposure to Cd on liver Pb content was exactly opposite. Markedly increased Pb residues in heart tissue of mice which received 2600 ppm Pb may be related to decreased virus-induced mortality in this group as mentioned above.

Pb and Cd each possess a divalent cation and are grouped together in the periodic table. Due to the similiarities of these metals, it is reasonable to assume that common pathways may be utilized for gastrointestinal absorption and transport within the body.

Decreased Pb residues in all tissues (except kidney) of mice coexposed to Cd could result from a general decrease in tissue binding sites for Pb in the presence of Cd. This would cause decreased accumulation of Pb in all tissues except possibly blood and kidney. The latter tissues could increase in Pb content due to decreased retension of Pb in other tissues and resultant increased transport of Pb via blood to the kidneys for excretion. An analogous reaction has been reported to occur after coexposure to Pb and selenium (Se). As the dose of Se was increased, tissue residues of Pb decreased, except in blood and kidney where Pb levels increased (Rastogi et al., 1976).

Kidney Pb residues were decreased in mice coexposed to 600 Cd-2600 Pb. The combined toxic effects of Pb and Cd on kidney cells at these high doses could result in an inability of these cells to synthesize protein which complexes with Pb to form the intranuclear inclusion bodies characteristic of Pb exposure. Inclusion bodies are a form of Pb storage in kidney cells. Inability to synthesize the protein necessary to complex Pb could result in decreased renal Pb storage. Alternate pathways of storage or excretion of Pb could be utilized which could account for increased Pb residues in the liver and testes of these mice. Increased concentrations of Pb in testes of mice treated with 600 Cd-2600 Pb could also be related to Cd-induced damage

of testicular vasculature, a lesion known to occur from Cd exposure. Destruction of blood vessels and consequent loss of vascular fluids adulterated with Pb into interstitial spaces, could increase Pb levels in the testes.

Increased Cd residues in tissues of mice coexposed to Pb could result from interference by Pb on metallothionine synthesis or binding of Cd. Pb interference could occur via damage to hepatocytes where metallothionine is synthesized. Impairment of metallothionine-Cd binding could result in increased "free" Cd in the blood that could accumulate in tissues to a greater degree than metallothionine-bound Cd. Decreased Cd residues in all tissues of mice treated with 600 Cd-2600 Pb could occur from a combined toxicity of Pb and Cd on kidney cells at these high doses causing decreased retension and increased excretion of Cd.

Decreased absorption of Pb due to competition with Cd for transport pathways or binding sites in the intestine could explain why Pb residues are decreased and Cd levels are increased in tissues of mice coexposed to these metals. Utilization of common intestinal absorption-transport pathways is indicated by alterations of Pb (Sorrell et al., 1977) or Cd (Thawley et al., 1977) intestinal absorption by calcium, vitamin D and zinc. Cd has been reported to reduce intestinal absorption of iron which indicates that gastrointestinal absorption of metal ions is affected by the presence of other metals (Prigge et al., 1977).

Histopathologic lesions produced by Pb and Cd in liver and kidney tissue of rats and rabbits indicate a potential for synergistic or

additive toxicity if exposed to these metals simultaneously. Ultrastructural alterations of hepatic cells after Pb or Cd exposure are identical in several respects. Both Pb (Hoffman et al., 1972) and Cd (Stowe et al., 1972) caused increased numbers of lysosomes which contain electron dense material thought to be a protein-metal complex, swollen mitochondria thought to result from inactivation of sulfhydryl-containing enzymes involved in cellular respiration, and dilation and fragmentation of smooth and rough endoplasmic reticulum.

Nephrotoxic effects of Cd (Fowler et al., 1975; Nishizumi, 1972) and Pb (Hammond, 1977) manifest as the Fanconi syndrome which is characterized by glycosuria, aminoaciduria and hypophosphatemia. These lesions are believed to be caused by damage to reabsorption mechanisms of the proximal convoluted tubules in the kidney cortex. Both Pb and Cd cause degenerative changes of these tubular epithelial cells.

Additive, synergistic or antagonistic effects in regard to tissue damage resulting from coexposure of mice to Pb and Cd were not evident in this study by light microscopy. Characteristic lesions of renal tissue in mice which have been reported previously (Koller et al., 1975; and Kovacic, 1974) were observed at the higher doses of each metal. Kidney damage consisted of moderate degeneration and necrosis of the tubular epithelial cells and flattening of Bowman's capsule resulting in an enlargement of Bowman's space and a smaller than normal appearance of the glomeruli. Kidney cells of Pb treated mice contained characteristic intranuclear inclusion bodies. Megakaryocytosis was observed in spleens of mice given Pb. This probably is indicative

of anemia caused by Pb exposure. Simultaneous exposure to the metals did not appear to alter these histologic lesions. Histopathologic changes that could be attributed to Pb or Cd exposure were not observed in any other tissues collected from these mice.

It is evident from mortality and tissue residue data obtained from this study that Pb and Cd interact in mammalian biological systems and alter effects produced by the individual metals. Additional research is required to determine the total effects, sites and mechanisms of interaction of Pb and Cd in mammals. Widespread environmental contamination by both metals and the potential for simultaneous exposure of humans and animals is indicative that further studies, not only of these metals, but contaminants in general, are imparative to understand the total effect of environmental pollution on human health.

TABLE I: EXPERIMENTAL DESIGN FOR EXPOSURE OF MICE TO LEAD AND/OR CADMIUM[a]

Diet (ppm)	Control	13 Pb	130 Pb	1300 Pb	2600 Pb
	No. of Mice Per Diet				
Control	15	15	15	15	15
3 Cd	15	15			
30 Cd	15		15		
300 Cd	15			15	
600 Cd	15				15

a) Pb and Cd were given in drinking water for ten weeks prior to inoculation (ip) with 0.2 ml of encephalomyocarditis virus diluted to $1x10^{-6.1}$.

TABLE II. PERCENT MORTALITY OF MICE EXPOSED TO LEAD AND/OR CADMIUM FOR TEN WEEKS PRIOR TO VIRUS CHALLENGE

Dose (ppm)		Final Mortality Rate (%)[ab]		
Lead	Cadmium	Lead	Cadmium	Lead & Cadmium
13	3	80 (12/15)[cd]	15 (2/13)[cd]	46 (6/13)
130	30	85 (12/14)[cd]	20 (3/15)[c]	47 (7/15)
1300	300	80 (12/15)[cd]	15 (2/13)[c]	36 (5/14)
2600	600	64 (9/14)[d]	40 (6/15)	25 (3/12)
Control		50 (7/14)	50 (7/14)	50 (7/14)

a) All mice were inoculated ip with 0.2 ml of encephalomyocarditis virus diluted to $1x10^{-6.1}$ after ten weeks exposure to lead and/or cadmium in the drinking water.

b) Mortality was recorded for 16 days after virus inoculation; no deaths occurred after day 13.

c) P = 0.05 to 0.07 significantly different from controls; Chi Square

d) P = 0.05 to 0.08 significantly different from lead and cadmium group; Chi Square

TABLE III: MEAN BODY WEIGHTS (GRAMS) OF MICE EXPOSED TO LEAD AND/OR CADMIUM[a]

Diet and Weeks of Exposure	Control	Metal Concentration (ppm) 3 Cd 13 Pb	30 Cd 130 Pb	300 Cd 1300 Pb	600 Cd 2600 Pb
		Body Weights (Mean ± Confidence Limit)			
0 Weeks					
Cd only		15.9 ± 0.9	16.0 ± 1.1	19.5 ± 0.9	15.7 ± 1.0
Pb only	16.1 ± 0.9	15.7 ± 1.1	16.1 ± 0.9	15.7 ± 0.9	16.1 ± 1.1
Cd + Pb		15.7 ± 1.1	15.6 ± 1.5	16.1 ± 1.2	16.0 ± 1.2
6 Weeks					
Cd only		35.6 ± 3.0	35.8 ± 3.1	32.7 ± 2.2	24.9[b] ± 1.8
Pb only	34.4 ± 2.7	40.4 ± 3.4	35.4 ± 2.3	39.5 ± 2.5	35.5 ± 2.7
Cd + Pb		36.9 ± 2.5	37.0 ± 2.4	29.4 ± 2.3	26.5[b] ± 2.5
10 Weeks					
Cd only		36.4 ± 2.7	40.4 ± 3.8	33.1 ± 3.0	23.6[b] ± 2.2
Pb only	37.3 ± 2.6	40.1 ± 1.7	35.9 ± 2.6	38.8 ± 2.2	33.4 ± 3.9
Cd + Pb		36.9 ± 2.1	37.1 ± 2.7	29.4[b] ± 2.7	26.1[b] ± 2.1

a) Lead and cadmium given via drinking water; ten mice per group

b) $P \leq 0.05$ to controls by 95% confidence limits

TABLE IV: CADMIUM RESIDUES IN TISSUES OF MICE EXPOSED TO CADMIUM OR CADMIUM AND LEAD IN THE DRINKING WATER FOR TEN WEEKS

ppm wet weight[a]

Diet (ppm)	Testes	Brain	Lung	Heart	Spleen	Liver	Kidney
Control	.02	.01	.04	0.2	.11	.26	.04
3 Cd	.04	.04	.04	.06	.16	1.18	3.17
3 Cd 13 Pb	.07	.06	.11	.16	.28	2.21	6.39
30 Cd	.36	.15	.88	1.18	2.26	18.11	26.35
30 Cd 130 Pb	.47	.16	.67	.88	1.81	26.68	49.27
300 Cd	1.05	.39	2.19	2.53	6.48	128.06	76.13
300 Cd 1300 Pb	1.55	.58	2.57	5.21	8.23	183.74	109.89
600 Cd	4.09	.92	6.35	7.08	21.40	282.09	127.94
600 Cd 2600 Pb	2.48	.55	5.77	6.82	16.53	178.73	110.08

a) Pooled samples of three mice per group

TABLE V: LEAD RESIDUES IN TISSUES OF MICE EXPOSED TO LEAD OR LEAD AND CADMIUM IN THE DRINKING WATER FOR TEN WEEKS

ppm wet weight[a]

Diet (ppm)	Testes	Brain	Lung	Heart	Spleen	Liver	Kidney
Control	.13	.12	.18	.71	.71	.55	.43
13 Pb	.39	.56	.74	.76	1.68	.85	1.56
13 Pb 3 Cd	.17	.09	.06	.30	.70	.70	3.06
130 Pb	.48	.52	1.03	.58	2.10	3.23	6.00
130 Pb 30 Cd	.16	1.10	.53	.18	1.98	2.02	8.39
1300 Pb	.95	4.21	5.71	.90	7.04	14.78	31.83
1300 Pb 300 Cd	.60	3.59	4.64	1.69	4.29	13.12	39.01
2600 Pb	1.89	6.29	8.28	5.00	16.47	11.20	46.68
2600 Pb 600 Cd	4.60	5.51	7.14	.89	8.11	44.08	33.11

a) Pooled samples of three mice per group

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